

Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing
Chicken Embryo-Report of the in-house investigations of **Cholic Acid** in the developing
chicken embryo 6/8/78

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

GRM18

TO : GRAS Review Branch, HFF-335


DATE: June 8, 1978

THRU: HFF-150 _____

FROM : Supervisory Chemist
Whole Animal Toxicology Branch (HFF-155)

SUBJECT: Investigation of the Toxic and Teratogenic Effects
of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the inhouse investigations of Cholic Acid
in the developing chicken embryo.


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Investigations of the Toxic and Teratogenic Effects of
GRAS Substances to the Developing Chicken
Embryo: Cholic Acid

Protocol:

Cholic Acid (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in acetone as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch.

Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 gave the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Cholic acid was administered up to 50 mg/kg at 0 hours by both routes, and at 96 hours up to 10 mg/kg and did not show any appreciable toxicity. No LD₅₀s could be calculated.

Scattered abnormalities were observed under all conditions of test, but serious abnormalities were not significantly higher than or different from those observed in the background. Cholic acid displayed no teratogenicity under the test conditions employed.

1. Cholic Acid, Lot #10350, Company unknown
2. McLaughlin, J., Jr., Marliac, J.P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol 5, 760-770
3. Verrett, M. J., Marliac, J.P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002-1006
4. Finney, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Cholic Acid
Air Cell at 0 Hours

Table 1

Dose		Number of Eggs	**Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
2.50	50.00	105	29.52	4.76	0.95
1.250	25.00	105	39.04	8.57*	0.00
0.50	10.00	105	34.28	2.85	1.90
0.250	5.00	104	33.65	0.96	0.00
0.1250	2.50	105	31.42	4.76	0.95
Acetone		105	36.19	0.00	0.00
Controls		299	21.07	2.34	0.33

*Significantly different from solvent $p \leq 0.05$

**Slope is negative

Cholic Acid
Air Cell at 96 Hours

Table 2

Dose		Number of Eggs	**Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
0.500	10.00	100	66.00	8.00*	2.00
0.250	5.00	100	65.00	6.00*	5.00*
0.100	2.00	99	65.65	4.04	2.02
0.050	1.00	100	62.00	7.00*	2.00
0.0250	0.50	129	49.61	3.87	2.32
Acetone		105	60.95	0.00	0.00
Controls		299	21.07	2.34	0.33

*Significantly different from solvent $p \leq 0.05$

**Slope is negative

Cholic Acid
Yolk at 0 Hours

Table 3

mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal	
				Total	Structural
2.50	50.00	105	58.09*	0.95	0.00
1.250	25.00	105	60.00*	0.95	0.00
0.50	10.00	105	58.09*	0.95	0.00
0.250	5.00	105	60.00*	0.95	0.95
0.1250	2.50	104	52.88*	0.96	0.00
Acetone		105	34.28	1.90	0.95
Controls		299	21.07	2.34	0.33

*Significantly different from solvent $p \leq 0.05$

**Slope not significantly different from zero $p = 0.05$

Cholic Acid
Yolk at 96 Hours

Table 4

mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal	
				Total	Structural
0.500	10.00	104	54.80*	1.92	0.00
0.250	5.00	105	60.95*	5.71	3.80
0.100	2.00	105	58.09*	3.80	2.85
0.050	1.00	105	53.33*	3.80	3.80
0.0250	0.50	75	56.00*	2.66	1.33
Acetone		109	36.69	1.83	1.83
Controls		299	21.07	2.34	0.33

*Significantly different from solvent $p \leq 0.05$

**Slope is negative